



# BIONETICS

Mutagenic Evaluation of Compound FDA 75-78  
Final Report 5/77

Thiamine HCT USP-FCC

P30

MUTAGENICITY EVALUATION

OF

FDA 75-78

THIAMINE HCT USP-FCC

000067-03-8

5516 Nicholson Lane  
Kensington, Maryland  
20795



P30

MUTAGENICITY EVALUATION

OF

FDA 75-78

THIAMINE HCl USP-FCC

000067-03-8

FINAL REPORT

SUBMITTED TO

DEPARTMENT OF HEALTH, EDUCATION AND WELFARE  
FOOD AND DRUG ADMINISTRATION  
NEGOTIATED CONTRACTS BRANCH  
5600 FISHERS LANE, HFA-510, 5B-37  
ROCKVILLE, MARYLAND 20852

SUBMITTED BY

LITTON BIONETICS, INC.  
5516 NICHOLSON LANE  
KENSINGTON, MARYLAND 20795

LBI PROJECT NO. 2672

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#### EVALUATION SUMMARY

The test compound, FDA 75-78, 000067-03-8, did not exhibit mutagenic activity in any of the assays employed in these studies.



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DATE: May 10, 1977

SPONSOR: U.S. Food and Drug Administration

SUBJECT: Evaluation of Test Compound FDA 75-78 Thiamine HCl USP-FCC  
000067-03-8

I. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

II. MATERIALS

A. Test Compound

1. Date Received: October 29, 1976
2. Description: White powder

B. Indicator Microorganisms

The following strains of indicator microorganisms were used in the evaluation:

Yeast Strain: Saccharomyces cerevisiae, strain D4

Bacteria Strains: Salmonella typhimurium, strains TA-1535  
TA-1537  
TA-1538  
TA-98  
TA-100

C. Reaction Mixture

The following reaction mixture was employed in the activation tests:

<u>Component</u>	<u>Final Concentration/ml</u>
1. TPN (sodium salt)	4 $\mu$ moles
2. Glucose-6-phosphate	5 $\mu$ moles
3. Sodium phosphate (dibasic)	100 $\mu$ moles
4. $MgCl_2$	8 $\mu$ moles
5. KCl	33 $\mu$ moles
6. Homogenate fraction equivalent to 25 mg of wet tissue.	



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D. Tissue Homogenates and Supernatants

The tissue homogenates and 9,000 x g supernatants were prepared from tissues of the following mammalian species: Mouse - ICR random bred adult males; rat - Sprague-Dawley adult males; and monkey - Macaca mulatta adult males.

E. Positive Control Compounds

Table 1 lists chemicals for positive controls in the direct and activation assays.

TABLE 1  
POSITIVE CONTROLS USED IN DIRECT AND ACTIVATION ASSAYS

<u>Assay</u>	<u>Chemical</u> <sup>a</sup>	<u>Solvent</u>	<u>Probable Mutagenic Specificity</u>
Nonactivation	Methylnitrosoguanidine	Water or saline	BPS <sup>b</sup>
	Ethylmethanesulfonate	Water or saline	BPS <sup>b</sup>
	2-Nitrofluorene	Dimethylsulfoxide <sup>c</sup>	FS <sup>b</sup>
	Quinacrine mustard	Water or saline	FS <sup>b</sup>
Activation	Dimethylnitrosamine	Water or saline	BPS <sup>b</sup>
	2-Acetylaminofluorene	Dimethylsulfoxide <sup>c</sup>	FS <sup>b</sup>
	8-Aminoquinoline	Dimethylsulfoxide <sup>c</sup>	FS <sup>b</sup>
	2-Aminoanthracene	Dimethylsulfoxide <sup>c</sup>	BPS <sup>b</sup>

<sup>a</sup> Concentrations given in the Results Section

<sup>b</sup> BPS = base-pair substitution; FS = frameshift

<sup>c</sup> Previously shown to be non-mutagenic

III. METHODS

A. Toxicity

The solubility, toxicity and doses for the test chemical were determined prior to screening.

The test chemical was tested for toxicity against specific indicator strains over a range of doses to determine the 50% survival dose. Bacteria were tested in phosphate buffer, pH 7.4, for one hour at 37°C on a shaker. Yeasts were tested in phosphate buffer, pH 7.4, for four hours at 30°C on a shaker. The 50% survival concentrations and the 1/4 and 1/2 50% doses calculated.

If no toxicity was obtained for the chemical with a given strain, then a maximum dose of 5% (w/v) was used.

Unless otherwise specified, the doses calculated for the tests in buffer were applied to the activation tests. The solubility of the test chemical under treatment conditions is stated in the Results Section.





## B. Plate Tests (Overlay Method)

Approximately  $10^8$  cells from an overnight culture of each indicator strain were added to test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For nonactivation tests, the three dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests 0.5 ml of a 9,000 x g tissue supernatant and required cofactors (core reaction mixture) were added to the overlay tubes. Three dose levels of the test chemical were added to the appropriate tubes, which were then mixed and the contents poured over the surface of a minimal agar (selective medium) plate and allowed to solidify. The plates were incubated for 48 to 72 hours at 37°C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using positive compounds that are active directly and those that require metabolic activation were run with each assay.

## C. Suspension Tests

### 1. Nonactivation

Bacteria and yeast cultures of the indicator organisms were grown in complete broth, washed and resuspended in 0.9% saline to densities of  $1 \times 10^{10}$  cells/ml and  $5 \times 10^9$  cells/ml, respectively. This constituted the working stock for tests of a group of test chemicals and their respective controls. Tests were conducted in plastic, 24-well tissue culture plates (Linbro). Cells plus appropriate volume(s) of the test chemical were added to the wells to give a final volume of 1.5 ml. The solvent replaced the test chemical in the negative controls. Treatment was at 30°C for four hours for yeast tests and at 37°C for one hour for bacterial tests. All flasks were shaken during treatment. Following treatment, the plates were set on ice. Aliquots of cells were removed, diluted in sterile saline (4°C) and plated on the appropriate complete media. Undiluted samples from flasks containing the bacteria were plated on minimal selective medium in reversion experiments. Samples from a  $10^{-1}$  dilution of treated cells were plated on the selected media for enumeration of gene conversion with strain D4. Bacterial plates were scored after incubation for 48 hours at 37°C. The yeast plates were incubated at 30°C for 3-5 days before scoring.

### 2. Activation

Bacteria and yeast cells were grown and prepared as described in the nonactivation tests. Measured amounts of the test and control chemicals plus 0.25 ml of the stock-cell suspension were added to wells of the Linbro plate containing the appropriate tissue fraction and reaction mixture. All flasks (bacteria and yeast) were incubated at 37°C with shaking. The treatment times as well as the dilutions, plating procedures and scoring of the plates were the same as described for nonactivation tests.



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D. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

Male animals (except monkeys) sufficient to provide the necessary quantities of tissues were killed by cranial blow, decapitated and bled. Monkey tissues were obtained from freshly killed and bled male rhesus monkeys. Organs were immediately dissected from the animals using aseptic techniques and placed in ice-cold 0.15M KCl. Upon collection of the desired quantity of organs, they were washed twice with fresh KCl and completely homogenized with a motor-driven homogenizing unit at 4°C. The whole organ homogenate obtained from this step was divided into two samples. One sample was frozen at -80°C and the other was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80°C. These two frozen samples were used for the activation studies. Protein and P-448 determinations were made for each lot of homogenate.

E. Data Recording and Reporting

1. Plate test assays

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were entered into a computer program designed to print out all data by test. The data are presented as revertants per plate for each indicator strain employed in the assay. The positive and solvent controls are provided as reference points.

2. Suspension assays

Following the specified incubation periods all population plates were scored by an automatic colony counter and the results from each plate of a set were recorded, in ink, on data processing forms. All minimal or other types of selective media plates were hand scored and the results recorded along with the respective population data. Other relevant experimental data were recorded on experimental definition forms. For bacteria strains the number of colonies recorded from either the population or selective plates represents that number in 1 ml of test suspension plated. The numbers recorded for the yeast strain D4 represent the number in 0.5 ml of test suspension plated. The data were then processed and printed from a computer program. All raw data sheets are dated and signed by the responsible technician.





IV. RESULTS SECTION

A. Solubility Properties of the Test Compound

1. Name or code designation of the test compound: 000067-03-8  
Thiamine HCl USP-FCC  
FDA 75-78
2. Test solvent: Saline
3. Solubility of the test compound under treatment conditions: Soluble
4. Additional comments: Clump white powder - chalk like consistency

B. Toxicity and Dosage Determinations for the Test Compound

1. Test date for toxicity determination:
2. The 50% survival level was determined for bacteria and yeast indicator organisms by conducting survival curves with the test compound at the following concentrations:

Percent Concentration (w/v or v/v)

5.0  
0.5  
0.05  
0.005  
0.0005

3. Concentrations of the test compound used in the mutagenicity tests:

<u>Test Doses</u>	<u>Percent Concentration</u>	
	<u>Bacteria</u>	<u>Yeast</u>
1/4 50% Survival	0.01825	0.002781
1/2 50% Survival	0.03650	0.05563
50% Survival	0.07300	0.1113



C. Plate Test Results

The plate test results are summarized in the following table. The values presented in this table are the number of revertants per plate.

D. Suspension Assay Results

The suspension test results for the test compound are summarized in the tables following the plate test summary. The values presented in these tables are the calculated mutation frequencies for each control and experimental test point. The first table of the suspension set presents the results for the nonactivation assays, and the second table through the fourth table of the suspension set presents the results for the activation assays. A listing of computer codes and abbreviations is included for reference. Tabulation of all raw data is provided in the Appendix.



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## SUMMARY OF TEST RESULTS

## PLATE TESTS

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: 000067038  
 B. TEST DATE: DEC. 16, 1976

TEST	SPECIES	ISSUE	B E V E R T A N I S P E R P L A T E									
			TA-1535		TA-1537		TA-1538		TA-98		TA-100	
			1	2	1	2	1	2	1	2	1	2
1. NON-ACTIVATION												
SOLVENT CONTROL*	---	---	24	34	17	15	18	21	53	47	122	189
POSITIVE CONTROL**	---	---	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
TEST 0.07300 %	---	---	29	35	18	15	21	14	43	39	132	138
0.03650 %	---	---	24	32	14	16	32	12	33	40	145	133
0.01825 %	---	---	33	34	17	15	22	19	42	35	120	150
2. ACTIVATION												
SOLVENT CONTROL*	MOUSE	LIVER	32	28	25	30	34	32	47	49	160	163
	RAT	LIVER	30	29	29	34	23	19	38	47	219	195
	MONKEY	LIVER	26	32	18	23	29	40	42	45	176	199
POSITIVE CONTROL***	MOUSE	LIVER	793	851	252	297	>1000	>1000	>1000	>1000	>1000	>1000
	RAT	LIVER	699	590	264	357	719	894	>1000	>1000	>1000	>1000
	MONKEY	LIVER	539	446	155	181	514	525	>1000	940	>1000	>1000
TEST 0.07300 %	MOUSE	LIVER	23	23	11	10	31	26	29	38	223	202
0.03650 %	MOUSE	LIVER	29	22	21	17	19	21	37	39	207	191
0.01825 %	MOUSE	LIVER	22	19	21	20	36	35	39	47	173	232
0.07300 %	RAT	LIVER	34	28	13	12	28	36	37	38	196	224
0.03650 %	RAT	LIVER	88	75	14	15	46	38	35	44	182	207
0.01825 %	RAT	LIVER	94	67	19	17	50	35	45	51	206	193
0.07300 %	MONKEY	LIVER	34	28	13	25	28	30	41	42	232	259
0.03650 %	MONKEY	LIVER	23	29	17	18	25	23	48	59	203	236
0.01825 %	MONKEY	LIVER	34	28	19	18	20	20	53	50	217	191

\* NON-ACTIVATION ASSAYS CONSIST OF THE CELLS PLUS THE TEST COMPOUND VEHICLE (SOLVENT). FOR ACTIVATION ASSAYS, THE OVERLAY CONTAINS THE ACTIVATION SYSTEM PLUS THE TEST COMPOUND VEHICLE.

\*\* TA-1535 MNNG 2 UG/PLATE  
 TA-1537 QM 20 UG/PLATE  
 TA-1538 NF 100 UG/PLATE  
 TA-98 NF 100 UG/PLATE  
 TA-100 MNNG 2 UG/PLATE

\*\*\* TA-1535 ANTH 100 UG/PLATE  
 TA-1537 AMQ 100 UG/PLATE  
 TA-1538 AAF 100 UG/PLATE  
 TA-98 AAF 100 UG/PLATE  
 TA-100 ANTH 100 UG/PLATE

NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS (UL) OR MICROGRAMS (UG) PER PLATE.

- INDICATES NO DATA WAS TAKEN.



LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
REPORT EX934

COMPOUND FREQUENCY SUMMARY REPORT 02/22/77

NONACTIVATION COMPOUND 000067038

TEST	ORG	TA100 HIS EX-8	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	000004 ADE EX-5	000004 TRY EX-5	
NAN		93.00	92.16	10.00	24.95	10.10	3.79	19.75	5.37	CONTROLS
NAP		891.53	1267.42	481.64	178.71	195.22	141.25	101.54	67.35	
NA1		35.71	85.46	5.45	20.92	10.71	3.61	10.35	5.61	TEST DATA
NA2		19.74	68.05	5.23	14.81	10.13	2.37	11.67	8.19	
NA3		36.88	92.83	7.17	14.61	10.64	2.93	7.82	5.95	



LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 02/22/77

SPECIFS ICRFLO/MOUSE COMPOUND 000067038

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	000004 ADE EX-5	000004 TRY EX-5	
ACT	A+C	184.08	4.97	5.44	7.54	3.58	14.04	11.23	NEGATIVE CONTROLS
ACT	A-C	70.56	5.02	4.77	10.87	2.69	16.96	13.67	
ACT	ALI	109.86	4.39	4.68	9.73	8.14	19.69	12.92	
ACT	ALU	78.31	4.92	5.66	10.04	5.45	20.41	15.61	
<hr/>									
ACT	PLT	251.33	119.24	141.85	222.79	64.02	76.42	76.94	POSITIVE CONTROLS
ACT	PLU	94.27	5.17	7.13	67.40	9.73	18.36	11.01	
<hr/>									
ACT	L11	73.51	9.98	6.65	15.30	8.30	12.94	4.85	TEST COMPOUND
ACT	L12	57.52	5.13	4.91	16.96	8.15	13.33	7.67	
ACT	L13	50.50	9.01	3.49	11.68	8.29	11.46	7.99	
ACT	LU1	76.11	4.41	5.57	16.85	8.03	7.73	5.23	
ACT	LU2	81.30	8.60	4.37	18.75	9.77	11.72	8.98	
ACT	LU3	100.00	9.48	3.70	14.47	6.38	12.78	8.08	



LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
REPORT EX934

COMPOUND FREQUENCY SUMMARY REPORT 02/22/77

SPECIES SPRDAN/RAT

COMPOUND 000067038

TEST	ORG	TA100 HIS EX-4	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	000004 ADE EX-5	000004 TRY EX-5
ACT	A+C	161.00	7.00	3.77	46.02	2.66	13.64	10.19
ACT	A-C	128.57	7.78	1.60	9.88	6.37	16.00	11.09
ACT	AL1	128.30	2.66	2.05	13.54	22.50	18.91	9.31
ACT	ALU	111.58	3.85	2.85	12.30	9.64	17.03	8.67
ACT	PLI	173.97	138.63	89.01	128.54	299.56	97.06	72.40
ACT	PLU	112.05	4.45	8.17	32.58	49.31	10.15	3.16
ACT	L11	99.16	8.57	3.78	12.94	9.60	14.91	6.91
ACT	L12	85.96	5.36	2.75	13.27	10.15	14.81	9.82
ACT	L13	104.95	3.80	5.60	16.23	10.00	9.86	5.87
ACT	LU1	105.31	5.86	3.68	15.41	10.09	12.13	7.33
ACT	LU2	100.21	4.23	2.68	14.97	9.27	12.95	11.22
ACT	LU3	95.28	2.46	2.43	17.65	11.07	12.60	10.34

NEGATIVE CONTROLS

POSITIVE CONTROLS

TEST COMPOUND



LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 02/22/77

SPECIES RHESUS/MONKEY COMPOUND 000067038

TEST	ORG	TA100 HIS EX-A	TA1535 HIS EX-A	TA1537 HIS EX-A	TA1538 HIS EX-A	TA98 HIS EX-A	000004 ADE EX-5	000004 TRY EX-5	
ACT	A+C	53.27	2.58	2.45	9.78	5.79	19.41	10.65	NEGATIVE CONTROLS
ACT	A-C	57.26	2.21	2.44	13.84	5.50	18.48	9.49	
ACT	ALI	82.47	3.39	2.07	12.29	9.56	16.49	10.91	
ACT	ALU	72.94	2.78	1.10	11.95	10.63	15.33	10.17	
ACT	PLI	195.14	36.71	81.50	137.05	123.31	93.48	73.43	POSITIVE CONTROLS
ACT	PLU	101.96	2.37	4.66	15.42	17.43	25.84	17.05	
ACT	L11	128.57	1.75	1.52	13.85	11.75	16.60	9.87	TEST COMPOUND
ACT	L12	101.33	2.92	1.42	11.26	11.90	13.95	10.53	
ACT	L13	72.41	2.80	1.81	15.01	13.14	21.43	9.74	
ACT	LU1	58.98	2.40	1.84	12.26	7.33	16.91	13.38	
ACT	LU2	41.01	2.02	2.13	15.03	10.20	23.64	11.50	
ACT	LU3	142.45	2.20	1.52	12.70	9.52	20.53	10.02	



## DATA TABLE TERMS AND ABBREVIATIONS

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
COMPOUND	Client designated compound number appears in this column.
TEST CODES	<p> NAN = Nonactivation: Solvent Control  NAP = Nonactivation: Positive Control  NA1 = Nonactivation: Test Compound Dose 1  NA2, etc. = Reflects the other dose level(s) </p> <p> A+C = Negative Chemical Control for ACP  A-C = Activation: Solvent Control  ALI or A+T = Activation: Homogenate Control (Liver)  ALU = Activation: Homogenate Control (Lung)  ACP = Activation: Positive Control  ACT = Activation Test </p> <p> LI = Liver Tissue Activation Fraction  LU = Lung Tissue Activation Fraction  KI = Kidney Tissue Activation Fraction  TE = Testes Tissue Activation Fraction  1,2, etc. = Dose Levels </p>
CONCENTRATION	<p>All test compound dose levels are expressed as a whole number followed by an exponent (negative) identified by the appropriate units.</p> <p>Example: 0025-2PCT = 0.25 percent concentration</p>
POPU	Total number of viable cells in the plating sample raised to some exponent printed directly below the abbreviation (i.e., EP + 6 = $\times 10^6$ ).
MUT 1	Total number of mutants or convertants obtained from the sample plated raised to some exponent printed directly below the abbreviation (i.e., EP + 0 = $10^0$ ). For strain D4, MUT 1 represents the number of ADE+ convertants.
MUT 2	Only used for strain D4 and represents the number of TRY+ convertants in the plated sample.
FREQ 1	The calculated mutation or gene conversion frequency times the negative exponent written directly below. For strain D4, FREQ 1 represents the ADE+ value.
FREQ 2	Only used for strain D4 and represents the TRY+ conversion frequency.
CONTAM	Presence of contamination on any plates.



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# DATA TABLE TERMS AND ABBREVIATIONS (continued)

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
AAF	2-Acetylaminofluorene
DMSO	Dimethylsulfoxide
DMN	Dimethylnitrosamine
EMS	Ethylmethanesulfonate
QM	Quinacrine Mustard
NF	Nitrofluorene
ANTH	2-Amino Anthracene
AMQ	8-Amino Quinoline
SPECIES	Animal Strains
SPRDAW	Sprague Dawley Rats
ICRFLO	Flow ICR Random Bred Mice
RHESUS	Rhesus Monkey ( <u>Macaca mulatta</u> )
MIXEDB	Dog, Mixed Breed
NEWZEA	New Zealand White Rabbit
UG	Microgram
UM	Micromole
ADE	Adenine
TRY	Tryptophan



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V. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound, FDA 75-78, 000067-03-8, was evaluated for genetic activity in a series of in vitro microbial assays with and without metabolic activation. The following results were obtained:

A. Salmonella typhimurium

1. Plate tests

The results of these tests were negative.

2. Nonactivation suspension tests

The results of these tests were negative. The test with TA-100 was repeated because of the high population counts at the NA1 and NA2 doses in the initial test.

3. Activation suspension tests

The results of these tests were negative.

B. Saccharomyces cerevisiae

1. Nonactivation suspension tests

The results of these tests were negative.

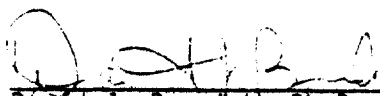
2. Activation suspension tests

The results of these tests were negative.

C. Conclusions

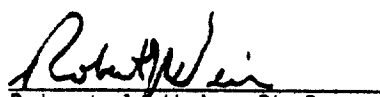
The test compound, FDA 75-78, 000067-03-8, did not exhibit mutagenic activity in any of the assays employed in these studies.

Submitted by:

  
David J. Brustick, Ph.D.  
Director  
Department of Genetics

5/11/77  
Date

Reviewed by:

  
Robert J. Weir, Ph.D.  
Vice President

5/11/77  
Date



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## VI. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and cells are incubated in the overlay for 2-3 days, and a few cell divisions occur during the incubation period, the test is semiquantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test.

- The small number of cell divisions permits potential mutagens to act on replicating DNA which is often more sensitive than non-replicating DNA.
- The combined incubation of the compound and the cells in the overlay permit constant exposure of the indicator cells for 2-3 days.

### A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs dose levels that are selected such that the highest dose will show slight toxicity (as determined by subjective criteria) and several doses ranging down 1 to 2 logs lower.

### B. Dose Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. Factors which may modify dose response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced and the compound will not appear to be mutagenic.

### C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.





#### D. Evaluation Criteria for Ames Assay

Because the procedures used to evaluate the mutagenicity of the test chemical are semiquantitative, the criteria used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets are evaluated using the following criteria:

##### 1. Strains TA-1535, TA-1537, and TA-1538

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

##### 2. Strains TA-98, TA-100, and D4

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-100 and two to three times the solvent control value for strains TA-98 and D4 is considered to be mutagenic. For these strains, the dose response increase should start at approximately the solvent control value.

##### 3. Pattern

Because TA-1535 and TA-100 were both derived from the same parental strain (G-46) and because TA-1538 and TA-98 were both derived from the same parental strain (D3052), there is a built-in redundancy in the microbial assay. In general the two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a given strain, e.g. TA-1537, responds to a mutagen in nonactivation tests it will generally do so in activation tests. (The converse of this relationship is not expected.) While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

##### 4. Reproducibility

If a chemical produces a response in a single test that cannot be reproduced in one or more additional runs, the initial positive test data loses significance.

The preceding criteria are not absolute and other extenuating factors may enter into a final evaluation decision. However, these criteria are applied to the majority of situations and are presented to aid those individuals not familiar with this procedure. As the data base is increased, the criteria for evaluation can be more firmly established.



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## VII. EXPLANATION OF EVALUATION PROCEDURES FOR SUSPENSION ASSAYS

Data obtained from mutagenicity tests are evaluated on a test by test basis followed by an examination of the total response pattern using all the data. To facilitate this type of evaluation, we have prepared two separate formats in which data are processed. The first is the Compound Summary Backup Detail Sheet, which details the essential raw data from each experiment showing surviving population counts, total mutant or revertant counts, as well as, calculated mutation frequencies. This format permits close examination of each set of test data. The following considerations are part of any assessment.

### A. Surviving Population Counts

A certain level of chemically-induced toxicity is anticipated, but occasionally isolated tests or groups of tests show very low (<25%) survival compared to the tissue controls. Such isolated decreases may result from improper dilution procedures or defective growth media and decrease confidence in the calculated mutation frequencies especially if the total mutant counts appear unaffected. Data of this type are generally unacceptable and these experiments are routinely repeated at a lower dose level to reduce killing and increase confidence in the nature of the response.

### B. Total Mutant Counts

For nonmutagens, the mutant/surviving population ratio should be roughly equivalent for each test point in a given experiment. If the cell number drops in response to killing, the mutant number should decrease proportionately. A mutagenic chemical, however, will produce an altered mutant/surviving population ratio. Mutant numbers as well as calculated frequencies are compared to the negative control data. In certain instances, the mutant frequencies will increase with little or no change in the absolute number of mutants especially where the test chemical is toxic. Data of this type, although not necessarily aberrant, or even rare, must be viewed with special care to ensure that the increased frequencies were not the result of selective toxicity of the test chemical for the his<sup>-</sup> cells. This phenomenon, referred to as selection, can lead to erroneous conclusions. Thus we attempt to keep the surviving population of cells high and look for positive responses that show increases in both numbers of mutants and mutation frequencies. Again, occasional isolated fluctuations in mutant counts are found that can be attributed to improper pipetting or media contamination. These fluctuations are usually easy to identify by inspection of the other data points in the experiment which will be negative.



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### C. Dose Response Phenomena

Dose-related increases in mutants and mutation frequencies are the most convincing data to have in assessing mutagenic activity of chemicals. In some cases, however, dose-related increases are not observed for mutagens. This depends considerably on the dose levels selected. The figure on the following page illustrates how one might obtain various types of dose-related responses by a mutagen based solely on dose selection. It also emphasizes the need to keep dose levels within a relatively low range of toxicity so that data are consistently on the uphill side of the hypothetical curve.

### D. Control Tests

Positive and negative control tests are conducted with each experiment and consist of direct acting positive agents for nonactivation assays and chemicals that require metabolic transformation for activation assays. In nonactivation assays, the NAN control contain the test chemical solvent plus cells, but no chemical, and is used as a reference to assess the level of response obtained in the various tests. It is not possible at this time to put precise cut-off points where negative responses become positive responses. A statistical component for our computer program is under development and will be included when available. Positive controls are only used as relative reference points and to demonstrate that the system is functioning with known mutagens. In activation assays, three types of negative controls are run: (1) A solvent control minus the chemical and minus the activation system (A-C); (2) a control plus the positive control chemical minus the activation system (A+C); and (3) a control containing the activation system and the test chemical solvent (ALI or ALU). All three controls are used collectively to assess the level of response in the various activation tests. A chemical may appear positive when compared to an A-C control but not when compared to an A+T control. The value of each of the above controls with respect to their weight in evaluation is  $ALI \text{ or } ALU > A-C > A+C$ .

The other data format is the Compound Frequency Summary Report sheet in which all the calculated frequencies obtained for a given compound are displayed in a table. This format permits an overview of all data. The points form a matrix of information that should present a consistent pattern. Nonmutagens should produce a matrix with data frequencies clustered around the negative control values. Occasional random high or low fluctuations are not uncommon and seldom indicate true genetic activity. Mutagenic chemicals should, on the other hand, produce a set of consistent responses that demonstrate a logical pattern. The patterns depend on the mutagenic specificity of the chemical but can be easily recognized in the Compound Frequency Summary Report format.

These mutagenicity assays are designed to optimize the probability of recognizing mutagens from nonmutagens and, in most cases, they work well. Occasionally, the data points are such that a definitive conclusion cannot be made without additional data.

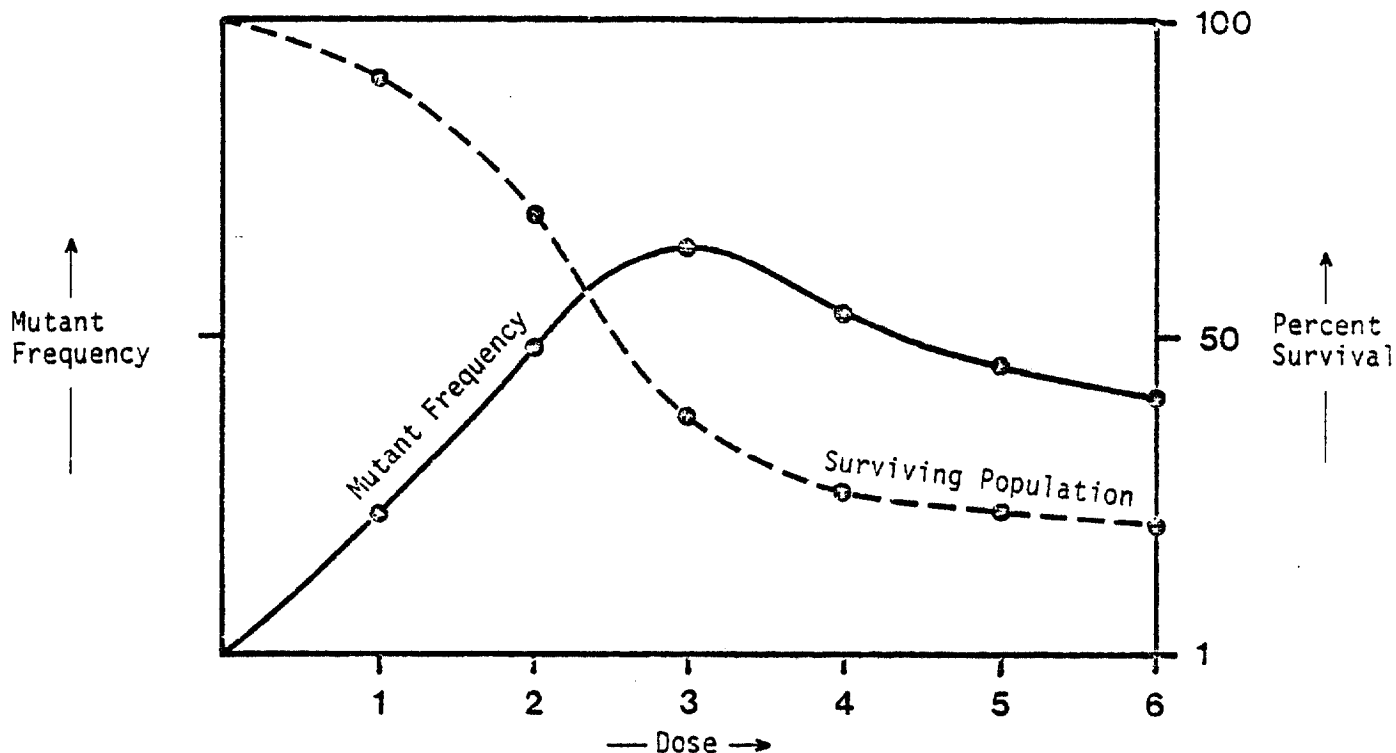


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# . HYPOTHETICAL MUTATION AND TOXICITY KINETICS



## HYPOTHETICAL EXPERIMENT

- (1) Dose levels 1, 2 & 3 were used
- (2) Dose levels 2, 3 & 4 were used
- (3) Dose levels 3, 4 & 5 were used

## OBSERVED DOSE RESPONSE

A typical positive dose response set of data would be obtained.

The intermediate dose level shows a higher mutation frequency than both the low dose and the high dose.

Here an inverted dose response would be observed with the highest dose level showing the lowest response.



APPENDIX  
Tabulation of Data



BIONETICS



REPORT EXP33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102		DETECTOR TA100		SPECIES		PROJECT 02672	DATE - 02/24/77
EXPERIMENT 704502						/	
COMPOUND	TFST	OPG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
		NAN	SOLVENT	0400	0376	92.16	0
		NAP	FMS 0.066%	0353	4474	1267.42	0
000067038	NA1		0073-3 PCT.	0227	0194	85.46	0
000067038	NA2		0365-4 PCT.	0457	0311	69.05	0
000067038	NA3		1825-5 PCT.	0516	0479	92.83	0



REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	633401	CONTRACT	22376-2102	DETECTOR	TA100	SPECIES	PROJECT	02672	DATE	- 02/24/77
COMPOUND	TEST	ORG ID	CONCENTRATION	POPUL	MUT1		FREQ1			
				EP+6	EP+0		EP-8		CONTAM	
		NAN	SOLVENT	0514	0470		93.00		0	
		NAP	EMS 0.066%	0744	6633		891.53		0	
000067038	NA1		0073-3 PCT.	1109	0396		35.71		0	
000067038	NA2		0365-4 PCT.	2346	0463		19.74		0	
000067038	NA3		1025-5 PCT.	0987	0364		36.88		0	



REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102		PROJECT 02672		DATE - 02/24/77			
EXPERIMENT 633702		DETECTOR TA1535		SPECIES /			
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	0480	0048	10.00	0
	NAP		EMS 0.2%	0828	3988	481.64	0
000067038	NA1		0073-3 PCT.	0808	0044	5.45	0
000067038	NA2		0365-4 PCT.	0841	0044	5.23	0
000067038	NA3		1825-5 PCT.	0753	0054	7.17	0



REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102		PROJECT 02672		DATE - 02/24/77			
EXPERIMENT 633406	DETECTOR TA1537	SPECIES	/				
COMPOUND	TFST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
		NAN	SOLVENT	1038	0259	24.95	0
		NAP	QM 13 UG/ML	0681	1217	178.71	0
000067038	NA1		0073-3 PCT.	0741	0155	29.92	0
000067038	NA2		0365-4 PCT.	1134	0168	14.81	0
000067038	NA3		1025-5 PCT.	1102	0161	14.61	0



REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102		PROJECT 02672		DATE - 02/24/77			
EXPERIMENT 633703		DETECTOR TA1538		SPECIES /			
COMPOUND	TEST	OP6 ID	CONCENTRATION	POP0 EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
		NAN	SOLVENT	0485	0049	10.10	0
		NAP	NF 667 UG/ML	0418	0816	195.22	0
000067038	NA1		0073-3 PCT.	0551	0059	19.71	0
000067038	NA2		0365-4 PCT.	0474	0048	19.13	0
000067038	NA3		1825-5 PCT.	0451	0048	10.64	0



REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102	PROJECT 02672	
EXPERIMENT 634120	DETECTOR 1A98	DATE - 02/24/77
	SPECIES	/
ORG	POPULATION	MUTATION
COMPOUND	TFST ID	CONCENTRATION
	EP+6	EP+0
	FREQ1	EP-8
	CONTAM	
NAN	SOLVENT	1397 0053 3.79 0
NAP	NF 667 UG/ML	1275 1801 141.25 0
000067038	NA1	0073-3 PCT. 1303 0047 3.61 0
000067038	NA2	0765-4 PCT. 1350 0032 2.37 0
000067038	NA3	1025-5 PCT. 1194 0035 2.93 0



REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672  
EXPERIMENT 634902 DETECTOR 000004 SPECIES / DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
		NAN	SOLVENT	0633	0125	0034	19.75	5.37	0
		NAP	FMS 1.0 %	0585	0594	0394	101.54	67.35	0
000067038	NA1		1113-4 PCT.	0802	0083	0045	10.35	5.61	0
000067038	NA2		5563-5 PCT.	0891	0104	0073	11.67	8.19	0
000067038	NA3		2781-6 PCT.	0908	0071	0054	7.82	5.95	0



REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672  
EXPERIMENT 633402 DETECTOR TA100 SPECIES ICRFL0/MOUSE DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POP EP+6	MUT EP+8	FREQ EP-8	CONTAM
	A+C		DMN 90 UM/ML	0201	0370	184.08	0
	A-C		SOLVENT	0591	0417	70.56	0
	ALI		TISSUE	0507	0557	109.86	0
	ALU		TISSUE	0747	0585	78.31	0
	ACP	LI	DMN 90 UM/ML	0450	1131	251.33	0
	ACP	LU	DMN 90 UM/ML	0524	0494	94.27	0
000067038	ACT	L11	0073-3 PCT.	0604	0444	73.51	0
000067038	ACT	L12	0365-4 PCT.	0572	0329	57.52	0
000067038	ACT	L13	1825-5 PCT.	0598	0302	50.50	0
000067038	ACT	LU1	0073-3 PCT.	0540	0411	76.11	0
000067038	ACT	LU2	0365-4 PCT.	0508	0413	81.30	0
000067038	ACT	LU3	1825-5 PCT.	0316	0316	100.00	0



REPORT EXP33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102		PROJECT 02672					
EXPERIMENT 634108		DETECTOR TAI535		SPECIES ICRFL0/MOUSE		DATE - 02/24/77	
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A-C		DMN 90 UM/ML	0945	0047	4.97	0
	A-C		SOLVENT	1096	0055	5.02	0
	ALI		TISSUE	1049	0046	4.39	0
	ALU		TISSUE	1118	0055	4.92	0
	ACP	LI	DMN 90 UM/ML	0421	0502	119.24	0
	ACP	LU	DMN 90 UM/ML	0831	0043	5.17	0
000067038	ACT	L11	0073-3 PCT.	0461	0046	9.98	0
000067038	ACT	L12	0365-4 PCT.	0429	0022	5.13	0
000067038	ACT	L13	1025-5 PCT.	0444	0040	9.01	0
000067038	ACT	LU1	0073-3 PCT.	0431	0019	4.41	0
000067038	ACT	LU2	0365-4 PCT.	0430	0037	8.60	0
000067038	ACT	LU3	1025-5 PCT.	0443	0042	9.48	0



REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672  
EXPERIMENT 701805 DETECTOR TA1537 SPECIES ICR/FLO/MOUSE DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FRFQ1 EP-8	CONTAM
	NAM		SOLVENT	0308	0004	1.30	0
	A+C		AMQ 333 UG/ML	0533	0029	5.44	0
	A-C		SOLVENT	0608	0029	4.77	0
	ALT		TISSUE	0449	0021	4.68	0
	ALU		TISSUE	0583	0033	5.66	0
	ACP	L1	AMQ 333 UG/ML	0466	0661	141.85	0
	ACP	L0	AMQ 333 UG/ML	0561	0040	7.13	0
000067038	ACT	L11	0073-3 PCT.	0737	0049	6.65	0
000067038	ACT	L12	0365-4 PCT.	0672	0033	4.91	0
000067038	ACT	L13	1825-5 PCT.	0687	0024	3.49	0
000067038	ACT	L01	0073-3 PCT.	0485	0027	5.57	0
000067038	ACT	L02	0365-4 PCT.	0442	0037	8.37	0
000067038	ACT	L03	1825-5 PCT.	0594	0022	3.70	0



REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672  
EXPERIMENT 634106 DETECTOR TA1538 SPECIES ICRFLO/HOUSE DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPUL EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0716	0054	7.54	0
	A-C		SOLVENT	0561	0061	10.87	0
	ALI		TISSUE	0514	0050	9.73	0
	ALU		TISSUE	0488	0049	10.04	0
	ACP	LI	ANTH 67 UG/ML	0531	1183	222.79	0
	ACP	LU	ANTH 67 UG/ML	0543	0366	67.40	0
000067038	ACT	L11	0073-3 PCT.	0523	0080	15.30	0
000067038	ACT	L12	0365-4 PCT.	0460	0078	16.96	0
000067038	ACT	L13	1025-5 PCT.	0488	0057	11.68	0
000067038	ACT	L11	0073-3 PCT.	0457	0077	16.85	0
000067038	ACT	L12	0365-4 PCT.	0448	0084	18.75	0
000067038	ACT	L13	1025-5 PCT.	0456	0066	14.47	0



REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672  
EXPERIMENT 634112 DETECTOR 1498 SPECIES ICRFLO/MOUSE DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	1945	0066	3.58	0
	A-C		SOLVENT	1671	0045	2.69	0
	ALI		TISSUE	0663	0054	8.14	0
	ALU		TISSUE	0734	0040	5.45	0
	ACP	LI	ANTH 67 UG/ML	0970	0621	64.02	0
	ACP	LU	ANTH 67 UG/ML	1387	0135	9.73	0
000067038	ACT	L11	0073-3 PCT.	0795	0066	8.30	0
000067038	ACT	L12	0365-4 PCT.	0687	0056	8.15	0
000067038	ACT	L13	1825-5 PCT.	0712	0059	8.29	2
000067038	ACT	L11	0073-3 PCT.	0719	0057	8.03	2
000067038	ACT	L12	0365-4 PCT.	0706	0069	9.77	0
000067038	ACT	L13	1825-5 PCT.	0800	0051	6.38	0



REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672  
EXPERIMENT 634805 DETECTOR 000004 SPECIES ICRFLO/MOUSE DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	0748	0105	0084	14.04	11.23	0
	A-C		SOLVENT	0578	0098	0079	16.96	13.67	0
	ALI		TISSUE	0650	0128	0084	19.69	12.92	0
	ALU		TISSUE	0593	0119	0091	20.41	15.61	0
	ACP	II	DMN 90 UM/ML	0396	0295	0297	76.42	76.94	0
	ACP	LU	DMN 90 UM/ML	0691	0125	0075	18.36	11.01	0
000067038	ACT	L11	1113-4 PCT.	0742	0096	0036	12.94	4.85	0
000067038	ACT	L12	5563-5 PCT.	0600	0080	0046	13.33	7.67	0
000067038	ACT	L13	2781-6 PCT.	0663	0076	0053	11.46	7.99	0
000067038	ACT	LU1	1113-4 PCT.	0841	0065	0044	7.73	5.23	0
000067038	ACT	LU2	5563-5 PCT.	0657	0077	0059	11.72	8.98	0
000067038	ACT	LU3	2781-6 PCT.	0681	0087	0055	12.78	8.08	0



REPORT EXR33 LITTON BIOMETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672  
EXPERIMENT 634206 DETECTOR TA1537 SPECIES SPRAW/RAT DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-B	CONTAM
	A+C		AMQ 333 UG/ML	1460	0055	3.77	0
	A-C		SOLVENT	1430	0023	1.60	0
	ALI		TISSUE	1075	0022	2.05	0
	ALU		TISSUE	1157	0033	2.05	0
	ACP	LI	AMQ 333 UG/ML	0382	0340	89.01	0
	ACP	LU	AMQ 333 UG/ML	1248	0102	8.17	0
000067038	ACT	L11	0073-3 PCT.	0661	0025	3.78	0
000067038	ACT	L12	0365-4 PCT.	0801	0022	2.75	0
000067038	ACT	L13	1825-5 PCT.	0500	0028	5.60	0
000067038	ACT	LU1	0073-3 PCT.	1113	0041	3.68	0
000067038	ACT	LU2	0365-4 PCT.	1158	0031	2.68	0
000067038	ACT	LU3	1825-5 PCT.	0947	0023	2.43	0



REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672  
EXPERIMENT 634208 DETECTOR T4100 SPECIES SPRDAW/RAT DATE - 02/24/77

COMPOUND	TFST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
		A+C	DMN 90 UM/ML	0441	0710	161.00	0
		A-C	SOLVENT	0644	0828	128.57	0
		ALI	TISSUE	0643	0825	128.30	0
		ALU	TISSUE	0717	0800	111.58	0
		ACP LI	DMN 90 UM/ML	0365	0635	173.97	0
		ACP LU	DMN 90 UM/ML	0581	0651	112.05	0
000067038	ACT	L11	0073-3 PCT.	0593	0588	99.16	0
000067038	ACT	L12	0365-4 PCT.	0527	0453	85.96	0
000067038	ACT	L13	1825-5 PCT.	0404	0424	104.95	0
000067038	ACT	LU1	0073-3 PCT.	0471	0496	105.31	0
000067038	ACT	LU2	0365-4 PCT.	0468	0469	100.21	0
000067038	ACT	LU3	1825-5 PCT.	0593	0565	95.28	0



REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672  
EXPERIMENT 703901 DETECTOR T41535 SPECIES SPRDAM/RAT DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0343	0024	7.00	0
	A-C		SOLVENT	0180	0014	7.78	0
	ALI		TISSUE	0527	0014	2.66	0
	ALI		TISSUE	0545	0021	3.85	0
	ACP	LI	DMN 90 UM/ML	0422	0585	138.63	0
	ACP	LU	DMN 90 UM/ML	0472	0021	4.45	0
000067038	ACT	L11	0073-3 PCT.	0210	0018	8.57	0
000067038	ACT	L12	0365-4 PCT.	0578	0031	5.36	0
000067038	ACT	L13	1825-5 PCT.	0843	0032	3.80	0
000067038	ACT	LUI	0073-3 PCT.	0324	0019	5.86	0
000067038	ACT	LU2	0365-4 PCT.	0732	0031	4.23	0
000067038	ACT	LU3	1825-5 PCT.	1096	0027	2.46	0



REPORT EXR33 LITTON BIOMETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672  
EXPERIMENT 635201 DETECTOR TA1538 SPECIES SPROAW/RAT DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0528	0243	46.02	0
	A-C		SOLVENT	0597	0050	9.08	0
	ALI		TISSUE	0539	0073	13.54	0
	ALU		TISSUE	0626	0077	12.30	0
	ACP	LI	ANTH 67 UG/ML	0515	0662	128.54	0
	ACP	LU	ANTH 67 UG/ML	0488	0159	32.58	0
000067038	ACT	L11	0073-3 PCT.	0487	0063	12.94	0
000067038	ACT	L12	0365-4 PCT.	0550	0073	13.27	0
000067038	ACT	L13	1025-5 PCT.	0530	0086	16.23	0
000067038	ACT	L11	0073-3 PCT.	0532	0082	15.41	0
000067038	ACT	L12	0365-4 PCT.	0561	0084	14.97	0
000067038	ACT	L13	1025-5 PCT.	0544	0096	17.65	0



REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672  
EXPERIMENT 634207 DETECTOR TA98 SPECIES SPRDAM/RAT DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-B	CONTAM
	A+C		ANTH 67 UG/ML	1729	0046	2.66	0
	A-C		SOLVENT	0973	0062	6.37	0
	ALI		TISSUE	0360	0081	22.50	0
	ALU		TISSUE	0643	0062	9.64	0
	ACP	LI	ANTH 67 UG/ML	0227	0680	299.56	0
	ACP	LU	ANTH 67 UG/ML	0720	0359	49.31	0
000067038	ACT	L11	0073-3 PCT.	0646	0062	9.60	0
000067038	ACT	L12	0365-4 PCT.	0542	0055	10.15	0
000067038	ACT	L13	1825-5 PCT.	0570	0057	10.00	0
000067038	ACT	LU1	0073-3 PCT.	0565	0057	10.09	0
000067038	ACT	LU2	0365-4 PCT.	0626	0058	9.27	0
000067038	ACT	LU3	1825-5 PCT.	0533	0059	11.07	0



REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672  
EXPERIMENT 634205 DETECTOR 000004 SPECIES SPRDAW/RAT DATE - 02/24/77

COMPOUND	TEST	OPB ID	CONCENTRATION	POPU EP-4	MUT1 EP-1	MUT2 EP-1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A-C		DMN 90 UM/ML	0638	0087	0065	13.64	19.19	0
	A-C		SOLVENT	0550	0088	0061	16.00	11.09	0
	ALI		TISSUE	0677	0128	0063	18.91	9.31	0
	ALI		TISSUE	0646	0110	0056	17.03	8.67	0
	ACP	LI	DMN 90 UM/ML	0442	0429	0320	97.06	72.40	0
	ACP	LU	DMN 90 UM/ML	0601	0061	0019	10.15	3.16	0
000067038	ACT	LI1	1113-4 PCT.	0637	0095	0044	14.91	6.91	0
000067038	ACT	LI2	5563-5 PCT.	0621	0092	0061	14.81	9.82	0
000067038	ACT	LI3	2781-6 PCT.	0835	0074	0049	8.86	5.87	0
000067038	ACT	LI1	1113-4 PCT.	0709	0086	0052	12.13	7.33	0
000067038	ACT	LI2	5563-5 PCT.	0633	0082	0071	12.95	11.22	0
000067038	ACT	LI3	2781-6 PCT.	0619	0078	0064	12.60	10.34	0



REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672  
EXPERIMENT 634209 DETECTOR TA100 SPECIES RHESUS/MONKEY DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0336	0179	53.27	0
	A-C		SOLVENT	0468	0268	57.26	0
	ALI		TISSUE	0485	0400	82.47	0
	ALU		TISSUE	0436	0318	72.94	0
	ACP	LI	DMN 90 UM/ML	0350	0683	195.14	0
	ACP	LU	DMN 90 UM/ML	0306	0312	101.96	0
000067038	ACT	L11	0073-3 PCT.	0294	0378	128.57	0
000067038	ACT	L12	0365-4 PCT.	0301	0305	101.33	0
000067038	ACT	L13	1825-5 PCT.	0424	0307	72.41	0
000067038	ACT	LU1	0073-3 PCT.	0490	0289	58.98	0
000067038	ACT	LU2	0365-4 PCT.	0673	0276	41.01	0
000067038	ACT	LU3	1825-5 PCT.	0212	0302	142.45	0



REPORT EXR13 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672  
EXPERIMENT 634902 DETECTOR TA1535 SPECIES RHESUS/MONKEY DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A-C		DMN 90 UM/ML	0854	0022	2.58	0
	A-C		SOLVENT	0905	0020	2.21	0
	ALI		TISSUE	1003	0034	3.39	0
	ALI		TISSUE	0792	0022	2.78	0
	ACP	11	DMN 90 UM/ML	0760	0279	36.71	0
	ACP	10	DMN 90 UM/ML	0930	0022	2.37	0
000067038	ACT	L11	0073-3 PCT.	1318	0023	1.75	0
000067038	ACT	L12	0365-4 PCT.	0959	0028	2.92	0
000067038	ACT	L13	1025-5 PCT.	1288	0036	2.80	0
000067038	ACT	L11	0073-3 PCT.	1084	0026	2.40	0
000067038	ACT	L12	0365-4 PCT.	1290	0026	2.02	0
000067038	ACT	L13	1025-5 PCT.	1089	0024	2.20	0



REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672  
EXPERIMENT 634210 DETECTOR T41537 SPECIES RHESUS/MONKEY DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+6	FREQ1 EP-8	CONTAM
	A+C		AMQ 333 UG/ML	2044	0050	2.45	0
	A-C		SOLVENT	1436	0035	2.44	0
	ALI		TISSUE	1784	0037	2.07	0
	ALU		TISSUE	2176	0024	1.10	0
	ACP	LI	AMQ 333 UG/ML	0454	0370	81.50	0
	ACP	LU	AMQ 333 UG/ML	1803	0084	4.66	0
000067038	ACT	L11	0073-3 PCT.	1772	0027	1.52	0
000067038	ACT	L12	0365-4 PCT.	1410	0020	1.42	0
000067038	ACT	L13	1825-5 PCT.	1328	0024	1.81	0
000067038	ACT	L11	0073-3 PCT.	1738	0032	1.84	0
000067038	ACT	L12	0365-4 PCT.	1600	0034	2.13	0
000067038	ACT	L13	1825-5 PCT.	1914	0029	1.52	0



REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102		PROJECT 02672					
EXPERIMENT 635102		DETECTOR TA1538		SPECIES RHESUS/MONKEY		DATE - 02/24/77	
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0501	0049	9.78	0
	A-C		SOLVENT	0549	0076	13.84	0
	ALI		TISSUE	0602	0074	12.29	0
	ALU		TISSUE	0569	0068	11.95	0
	ACP	LI	ANTH 67 UG/ML	0529	0725	137.05	0
	ACP	LU	ANTH 67 UG/ML	0493	0076	15.42	0
000067038	ACT	L11	0073-3 PCT.	0462	0064	13.85	0
000067038	ACT	L12	0365-4 PCT.	0462	0052	11.26	0
000067038	ACT	L13	1825-5 PCT.	0473	0071	15.01	0
000067038	ACT	LU1	0073-3 PCT.	0514	0063	12.26	0
000067038	ACT	LU2	0365-4 PCT.	0439	0066	15.03	0
000067038	ACT	LU3	1825-5 PCT.	0441	0056	12.70	0



REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 72376-2102		PROJECT 02672					
EXPERIMENT 634302	DETECTOR TA98	SPECIES RHESUS/MONKEY				DATE - 02/24/77	
COMPOUND	TFST	ORG ID	CONCENTRATION	POPJ EP+6	MUTJ EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	2229	0129	5.79	0
	A-C		SOLVENT	1691	0093	5.50	0
	ALI		TISSUE	0973	0093	9.56	0
	ALU		TISSUE	1355	0144	10.63	0
	ACP	LI	ANTH 67 UG/ML	1137	1402	123.31	0
	ACP	LU	ANTH 67 UG/ML	1308	0228	17.43	0
000067038	ACT	L11	0073-3 PCT.	1302	0153	11.75	0
000067038	ACT	L12	0365-4 PCT.	1168	0139	11.90	0
000067038	ACT	L13	1825-5 PCT.	1301	0171	13.14	0
000067038	ACT	LU1	0073-3 PCT.	1597	0117	7.33	0
000067038	ACT	LU2	0365-4 PCT.	1510	0154	10.20	0
000067038	ACT	LU3	1825-5 PCT.	1491	0142	9.52	0



REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672  
EXPERIMENT 701806 DETECTOR 000004 SPECIES RHESUS/MONKEY DATE - 02/24/77

COMPOUND	TFST	ORG ID	CONCENTRATION	POPU EP-4	MUT1 EP-1	MUT2 EP-1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
		NAN	SOLVENT	0357	0101	0000	28.29	0.00	0
		A+C	DMN 90 UM/ML	0742	0144	0079	19.41	10.65	0
		A-C	SOLVENT	0790	0146	0075	18.48	9.49	0
		ALI	TISSUE	0770	0127	0084	16.49	10.91	0
		ALI	TISSUE	0698	0107	0071	15.33	10.17	0
		ACP	LI DMN 90 UM/ML	0414	0387	0304	93.48	73.43	0
		ACP	LU DMN 90 UM/ML	0387	0100	0066	25.84	17.05	0
000067038	ACT	LI1	1113-4 PCT.	0699	0116	0069	16.60	9.87	0
000067038	ACT	LI2	5563-5 PCT.	0731	0102	0077	13.95	10.53	0
000067038	ACT	LI3	2781-6 PCT.	0616	0132	0060	21.43	9.74	0
000067038	ACT	LU1	1113-4 PCT.	0680	0115	0091	16.91	13.38	0
000067038	ACT	LU2	5563-5 PCT.	0626	0148	0072	23.64	11.50	0
000067038	ACT	LU3	2781-6 PCT.	0599	0123	0060	20.53	10.02	0